ANNUAL REPORT OF COOPERATIVE MULTISTATE PROJECTS

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PROJECT: Multistate Research Project S-009

Plant Genetic Resources Conservation and Utilization

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PROGRESS OF THE WORK AND PRINCIPAL ACCOMPLISHMENTS:

Seed Storage and Database Management:

Genetic resources representing 902 new accessions were received, increasing the total collection maintained at the Plant Genetic Resources Conservation Unit (PGRCU) to 80,097 accessions. The new introductions represented a broad range of five families, fourteen genera, and thirty-eight species from thirty-four countries. A total of 1,350 accessions were requested for regeneration from S-9. Thus far, 507 inventory samples that were harvested in 1999 have been processed into cold storage. This includes 445 pearl millet accessions which were sent from the National Seed Storage Laboratory (NSSL) to St. Croix for quarantine increase.

There were 630 orders processed containing 16,027 items (in-vitro, plants, rhizomes, seeds, canes) with 27% (4,304 accessions) of the order items being supplied to foreign requestors. More than 2,400 items were shipped to the NSSL for long-term storage or backup. These included peanuts (687 accessions), watermelon (30), cucurbits (21), hibiscus (18), grasses (624), sorghum (712), and *Vigna* (289). Overall, 72.7% of the collection has been backed up at NSSL.

In complement, 193,079 records were created and 210,709 records were modified by site personnel to enhance the Germplasm Resources Information Network (GRIN) database. Evaluation studies and associated observation data were added to the GRIN for clover, peanuts, peppers, cowpea, luffa, and sorghum. Traits evaluated included disease, plant growth, insect damage, molecular, morphology, phenology, production, quality, stress, and taxonomic. A total of 134,964 observation records were created or modified for these studies. Characterizations were also obtained in all regenerations conducted by the curators. Training was begun and is ongoing with two employees in using the GRIN to maintain passport and observation data, as well as process orders and field books.

Numerous accessions (1,138) of annual clover and special-purpose legumes were sent to researchers throughout the world for use on forage, archaeology, breeding programs, identity

^{*} Indicates a voting member of the Technical Committee

checks, genetic resources, teaching, salinity reduction studies, winter hardiness, powdery mildew resistance, crop evaluations, physiology, restoration, phylogenetics, new crop evaluation, taxonomy, human diet, soil improvement studies, genome diversity, edible legumes, ornamental research, seed production, revegetation, farming systems, virus resistance, cover cropping, population structures, natural products, genetic improvement as well as both nutraceutical (16 accessions) and pharmaceutical (28 accessions) studies for human therapeutic applications.

The existing bar code system was enhanced with the addition of a workstation to produce ondemand shipping labels for orders as well as aiding with in-house processing. It includes an offline database accessible through a single form-based interface on a PC. The use of bar codes and related systems has increased efficiency and accuracy while enabling a smaller staff to process and distribute germplasm in a timely manner. Equipment was purchased for a workstation to be set up in another seed storage work room.

An S-9 Multistate Research Project web site (http://www.ars-grin.gov/ars/SoAtlantic/Griffin/pgrcu/s9.html) was developed and linked to the ARS and PGRCU web site. The S-9 Project web site provides an easily accessible system and common repository for information such as membership lists, abbreviated history, project objectives, minutes, annual reports, publications, and announcements associated with the multistate research project.

An extensive Y2K testing and remediation project was begun in late 1998 and completed in 1999. In conjunction, communication to the GRIN was upgraded with a Frame Relay circuit, and a new compliant router was purchased with Y2K funding. Also, a detailed security plan was completed for the Unit; including telecommunications, computer equipment, and overall of the facilities.

Curatorial Regeneration:

Clearance was obtained and procedures were established to grow Pearl Millet under quarantine conditions at St. Croix after two years of negotiation with APHIS. A compilation of information of the known pathogens of Pearl Millet was developed by Dr. Jeff Wilson, ARS, Tifton, Georgia which accelerated the APHIS processing of the quarantine permit.

The project of cleaning, weighing, and counting the warm season grasses was completed. This collection consists of more than 6,700 accessions in 101 genera and almost 500 species. At Griffin, 161 grass, 31 castor, 12 sesame, and 6 waterchestnut accessions were regenerated. In addition, 30 Hibiscus spp. and 637 quarantined pearl millet accessions from the winter nursery at St. Croix were cleaned and processed into the collection. An additional 98 bamboo plots at Byron, Ga were maintained.

A total of 53 cowpea lines which had not produced seeds in the field were regenerated in the greenhouse, 51 of which produced seeds. For field increase at Griffin, 153 cowpea lines were planted in the greenhouse to check for virus symptoms before transplanting to the field. Of these

lines, two failed to germinate, 28 were grown in the greenhouse because they were last seed or had poor germination and 123 were taken to the field for regeneration. Only 84 lines produced seed in the field and 26 lines in the greenhouse. In addition, six lines that had not produced seed in Georgia were grown in the field in Puerto Rico in the summer and all six produced seed. This left 41 lines which produced plants but no seeds; they will have to be grown either in the field in Puerto Rico or in the greenhouse. Descriptive data were collected on all increases and entered into GRIN.

A new growth chamber was installed in Bldg. 4457 (Plant Genetic Resources Laboratory) to house the in vitro sweetpotato collection. This upgrade in facilities greatly improves the security of the collection. Seven hundred fifty sweetpotato accessions maintained on nutrient agar were clonally regenerated.

Field regenerations of vegetables included 100 accessions of watermelon, 6 miscellaneous accessions of curcurbits, and 50 accessions of okra. However, due to a herbicide carry-over problem, very few okra seed were obtained. The increase samples of the pepper collection were cleaned and work was begun on weighing, counting, bar coding, and backing up the samples.

Thirty-four self-pollinated annual clovers, 33 cross-pollinated annual clovers, 20 Ethiopian clovers, 28 Winged bean, 7 Serradella, and 123 legumes were regenerated in 1999. Forty-seven legumes were reordered due to low viability. In all, 292 clover and minor legume accessions were regenerated in 1999. The Ethiopian clovers and the winged bean are Fall annuals and are continuing regenerating this FY in a greenhouse. A major lectin producing legume species, *Canavalia ensiformis* was successfully regenerated in the field by transplanting older plants to the field in June when the soil temperatures had increased sufficiently. Crimson clover accessions were regenerated by direct seeding in the fall, resulting in quality crimson clover regeneration. In addition, velvetbean (*Mucuna pruriens* var. *utilis*), a major phytopharmaceutical/nutraceutical species, was successfully regenerated by utilizing a freeze protectant known as remay fiber. This remay fiber sheet was placed over plants with immature pods in October. Healthy and unfrozen quality seed were later harvested from several of these velvetbean accessions. Thus far this winter, exceptional quality early growth was observed for several *Ornithopus* spp. (Serredella) and *Trifolium* spp. from both direct seeding and transplants at the PGRCU regeneration plots at Byron, GA.

Over 500 cultivated peanut accessions were regenerated at Byron, Ga in 1999. Ten accessions of wild peanut species were regenerated at Griffin, Ga. Associated characteristic data were recorded for all regenerated accessions.

Curatorial Research:

Seven peanut SSR markers were used to evaluate the within accession variation and to determine if cultivars could be separated at the varietal level of classification as proposed by Krapovickas and Gregory in a selected group of cultivated peanut accessions. Accessions evaluated were 25

Arachis hypogaea var. hirsuta, 12 A. hypogaea var. peruviana, and two each from A. hypogaea var. hypogaea, vulgaris, fastigiata, and aequatoriana. All SSR markers were polymorphic except for one (Ah6-125). The phenogram derived from the SSR data seems to indicate that the accessions cluster together better according to geographical location rather than botanical variety. For example, one clade was comprised solely of accessions collected from the state of Pinchincha, Ecuador within one degree area of both longitude and latitude. Three other clades were comprised of accessions from Mexico, each clade representing different, confined areas within the country. No clear segregation of peanut variety into clades was apparent. This suggests that more genetic variability may be found by selecting from different geographic areas rather than basing selection on botanical variety, and may indicate a need for changing the composition of the core collection. Many accessions had very low within accession variation based on the SSR data, a desired characteristic as it indicates that the homogeneity of an accession is high. There were some accessions, however, that were not genetically homogenous and may represent mixed seed. It is imperative for peanut curation that more SSR markers be discovered in order to maximize the discerning power of the marker set thus improving the core collection.

An improved method for the detection of two viruses in peanut seeds, peanut stripe virus and peanut mottle virus, has been developed. This method, called immunocapture-reverse transcription-polymerase chain reaction, is much more sensitive than currently used serological methods for detecting peanut stripe and peanut mottle viruses in seeds. This method allows for large numbers of seed to be processed more rapidly than with currently used serological test and reduces the chance of these viruses being brought into the U.S. or being distributed to peanut growing areas

Cultivated and wild peanut germplasm was identified with resistance to thrips, tomato spotted wilt virus, early maturation, and/or good yield. This identification of new cultivated and wild peanut germplasm with improved characteristics offers the breeder new sources of genes which will broaden the genetic base for cultivated commercial peanuts.

A core collection for sorghum (2,443 accessions) was identified, bringing the total number of crops with core collections to seven, clover (three species: *T. alexandrinum, T. resupinatum, T. subterraneum*), cultivated peanut, eggplant, okra, mung bean, cowpea, and sorghum.

Major accomplishments with vegetable crops included: the isolation and sequencing of 100+ microsatellite loci from pepper (*Capsicum annuum*), further characterization of the activation of retrotransposons in sweetpotato, and a preliminary screen of the *Cucurbita moschata* germplasm from Mexico for genetic diversity.

Through collaborative efforts, we discovered that *Canavalia ensiformis, Indigofera spicata*, and *I. nummularifolia* reduce root-knot nematode galls by 90% when added as a soil amendment. Thus *C. ensiformis, I. spicata*, and *I. nummularifolia* could be used as a rotation crop in the southeastern U.S. or used in home gardens for control of *Meloidogyne* spp. nematode

populations. Furthermore, additional legume species including *Clitoria ternatea*, and *Desmodium adscendens* have been identified as having useful phytochemicals and quality plant production characteristics at Griffin, GA. Several *Crotalaria* spp. have been identified as having either additional or newly identified phytochemicals.

Applied Genetics Analysis Laboratory:

An upgrade of our PE Applied Biosystems 373 sequencer was purchased, installed, and is now in operation which will allow us to double our sequencing output. The Applied Genetic Analysis Lab has made its priority to assists the curators with their respective molecular needs. The current personnel is as follows 1- PhD bioinformatics / statistics scientist, 2 MS research coordinators and 1- hourly worker. Despite having lost two research scientists (Research Leader and Lab Coordinator) and a very skilled laboratory technician, the Applied Genetics Analysis Lab has made accomplishments with curatorial needs. We are nearly halfway through a two-year study accessing variability in subterranean clover. We also have completed an initial study to assess SSR markers in peanut for their ability to discriminate cultivated accessions, and begun efforts to find more SSR markers in peanut. Also, we have provided sequencing of over 500 cDNA clones for sweetpotato. The Applied Genetic Analysis Lab has also worked with scientists outside of our department. We have provided training and assistance to the following: Dr. Dave McMillin of Clark Atlanta University, Dr. Jerry Johnson-s PhD student of the University of Georgia, visiting scientist Marta Martini from Italy, and Dr. Tracie Jenkins of the University of Georgia. The applied genetic analysis lab has also made suggestions for future studies based on their perception of trends in molecular genetics and population studies. We have suggested a large-scale sequencing project to sequence cDNA clones from crops located in our unit. This would benefit the unit two-fold: first it would provide molecular markers for genetic characterization of related crop material, and second it would provide a sequence database possibly revealing important traits by comparison to know cDNA sequences.

USEFULNESS OF FINDINGS:

The germplasm collections maintained at the PGRCU are critically important as a source of genetic material for plant breeders to develop new and improved plant varieties that will assure an abundant supply of high quality food, fiber and new value-added products including phytopharmaceuticals, nutraceuticals and pesticidal phytochemicals for our nation. Collaborative efforts among scientists at the regional repository, other federal laboratories, state agricultural experiment stations, and industry are mutually beneficial as sources of research information and products that are available for all customers. Through the efforts at the repository, broad genetic representation of crop plants and their weedy/wild relatives is maintained for ready access. The identification of desirable traits among accessions aid in crop improvement efforts to produce a higher quality product more efficiently with innate resistance to pathogens or insects and in a more environmentally friendly, sustainable manner. Information gained from cropping system studies of potential new crops may lead to greater diversification of agriculture in the southeastern United States. Furthermore, development of core collections and molecular markers

to determine diversity/redundancy will assist in reducing costs of maintaining germplasm collections.

STATEMENT OF ACCOMPLISHMENTS:

In 1999, there were 630 orders processed containing 16,027 items (in-vitro, plants, rhizomes, seeds, canes) with 27% (4,304 accessions) of the order items being supplied to foreign requestors. More than 2,400 items were shipped to the NSSL for long-term storage or backup. These included peanuts (687 accessions), watermelon (30), cucurbits (21), hibiscus (18), grasses (624), sorghum (712), and *Vigna* (289). Overall, 72.7% of the collection has been backed up at NSSL. These requested and evaluated genetic resources included traits used for disease resistance, insect resistance, improved quality, and phytochemicals with useful therapeutic properties in recently released lines of sorghum, peanut, watermelon, cowpea, grasses, pepper, and other commodities. In complement, 193,079 records were created and 210,709 records were modified by site personnel to enhance the Germplasm Resources Information Network (GRIN) database. Evaluation studies and associated observation data were added to the GRIN for clover, peanuts, peppers, cowpea, luffa, and sorghum. Traits evaluated included disease, plant growth, insect damage, molecular, morphology, phenology, production, quality, stress, and taxonomic. A total of 134,964 observation records were created or modified for these studies. Characterizations were also obtained in all regenerations conducted by the curators.

Seven peanut SSR=s indicated that the accessions are more related with regard to geographical location rather than botanical variety, and suggest that more genetic variation in the core collection may be obtained by selection by diversity of geographical areas.

A core collection of 2,443 accessions of sorghum was identified.

An improved method for detection of peanut stripe virus and peanut mottle virus in seeds was developed.

Cultivated and wild peanut germplasm was identified with resistance to thrips, tomato spotted wilt virus, early maturation, and/or excellent yield.

Over 100 microsatellite loci from Capsicum annuum were isolated and sequenced.

An upgrade of our PE Applied Biosystems 373 sequencer has doubled our sequencing output in the Applied Genetic Analysis Laboratory.

WORK PLANNED FOR FY2000:

Vigna:

1. Regenerate a minimum of 300 Vigna accessions for seed increase and characterization, 50

or more of which may have to be increased in the greenhouse (for photoperiod reasons). The seeds obtained from the winter increase will be used for a summer increase at Puerto Rico. A group of ~50 lines of photoperiod-sensitive materials will be increased at Puerto Rico.

2. Plan and perform tests to characterize several unknown viruses isolated from crops in regeneration. The most important of these is a virus(es) of *Crotalaria* which seems to be seedborne and is very severe on tobacco, but which has defied attempts at identification thus far. Biological tests, serology, dsRNA, purification, and electron microscopy have given few positive results to date. This has been a cooperative effort with laboratories at Tifton, Ga, Beltsville, Md, and with the special -purpose legume curator at Griffin.

Peanut:

- 1. Five hundred to 1000 cultivated peanuts will be grown in Byron, Ga., for increase. Selection will be made based on low seed counts and then lack of back up at Ft. Collins at NSSL.
- 2. Approximately, 10 wild peanut accessions will be selected for increase in the greenhouse. Each accession will be grown in about 20 baskets or trays, so that sufficient seed is returned for storage and backup.
- 3. Approximately 350 cultivated peanuts from China, Ecuador, Guatemala will be evaluated for virus and other disease resistance and pest resistance at Attapulgus, Ga. Disease and pest notes will also be made available for entry into GRIN.
- 4. Bayo Grande and five Bayo Grande-like accessions will be compared with selected varieties available to the growers now and evaluated for yield and disease and pest resistance Attapulgus, Ga.
- 5. Five to fifteen wild species will be increased outside at Griffin, Ga., in cages and without cages using bumble bees to aid pollination. The purple variety of *Echninacea*, ornamental cone flower, will be used to attract the bees for pollination.
- 6. Develop a genomic library for cultivated peanut and screen the library for SSRs. We will develop markers based on the results of the screening and test the markers to determine if they are polymorphic. We have submitted a proposal to the Georgia Agricultural Commodity Commission for Peanuts in hopes of providing funds for the project.
- 7. Screen an *Arachis* molecular core collection, approximately 80 accessions, using SDS-PAGE to document the ability of the protein profiles generated in distinguishing among the accessions at the botanical variety level. Subsequently, a western blot will be performed using serum from peanut allergic patients to see if any differences exist among

cultivars in the binding of IgE to proteins, i.e., to test for differences in allergens present in the peanut. A proposal will be submitted to the American Peanut Foundation in hopes of providing funds for this project.

- 8. Use the seven SSR markers available to date to screen 45 accessions of cultivated peanut. This work is a continuation of a project funded by the Georgia Commodity Commission for Peanuts in 1999. The project as funded has been completed, but the results demonstrate a need to grow individual plants out in the field and couple morphological data gathered in the field with molecular data generated using the SSR marker set for each plant individually. Basically, the plants will be grown out in the spring, morphological data will be collected for each individual plant, DNA will be extracted for the leaf material of each plant and SSR profiles will be generated using this DNA.
- 9. Plan and perform tests to develop PCR-based detection method for the strain of cowpea aphid-borne mosaic virus (CABMV) of peanuts from Brazil. Primers that we have already designed based on the 3' sequence data obtained by cooperators in Tifton, GA will continue to be tested to maximize the conditions. The primary approach left to develop is a way to use the IC-RT-PCR method for CABMV so that it can be used in tandem or in a multiplexing approach with the primers of PStV and PeMV. This work is being done through cooperation with the UGA virology lab at Tifton, GA.
- 10. Plan and perform tests to develop a RT-PCR test for detection of peanut clump furovirus in peanuts from Africa and India. This will involve development of procedures for inoculation of peanuts with the strains of the virus that we have imported so that extraction methods can be developed for peanuts and not just tobacco. Obtaining some additional strains of the viruses would also add to our knowledge of how the method will work.
- 11. In cooperation with the USAID Peanut Collaborative Research Support Program crossing of wild by wild and cultivated by cultivated peanut will continue with the purpose of developing new varieties with improved resistance for disease and pests for use in the United States.

Vegetables:

- 1. Increase and characterize a minimum of 200 germplasm accessions which will include 100 accessions of *Citrullus lanatus* and other vegetable crops germplasm.
- 2. Plan and conduct research relative to the utilization of molecular markers for characterization of plant germplasm including characterization of diversity in *Cucurbita moschata*.
- 3. Continue investigation into molecular basis of retrotransponson activation in vitro. Isolate

and sequence molecular markers from vegetable crops germplasm.

4. Maintain 7,000 tissue cultures of sweetpotato and distribute on request.

Minor Legumes:

- 1. Regenerate 300 annual clovers and legumes during FY 00.
- 2. Conduct field tests to identify particular cropping systems needed to optimally regenerate quality seed and to determine if particular species such as phytopharmaceutical/nutraceutical species can and will grow well in Griffin and/or Byron.
- 3. Identify gaps in the GRIN system and correct.
- 4. Identify additional phytopharmaceutical/nutraceutical/pesticidal legume species within the annual clover and special-purpose legume collection.
- 5. Complete molecular marker (AFLP) discovery for identifying variation/duplication within the *Trifolium subterraneum* collection.
- 6. Conclude a collaborative study on the effect of various legume species as soil amendments for suppression of root-knot nematode with Jerry Walker, plant pathologist, UGA, Griffin, GA.
- 7. Conduct a collaborative study regarding potential nematicidal activity in accessions of *Canavalia ensiformis* in comparison with natural lectins (Con-A) with Jerry Walker.
- 8. Collaborate with Barry Cumfer, plant pathologist, UGA, Griffin, GA in the evaluation of *T. subterraneum* for powdery mildew resistance.

Grasses and Others:

- 1. Five hundred PI=s of aged and/or low seed quantities will be grown for regeneration of seed on a priority basis as determined by analysis of the detailed inventory. A minimum of 300 new accessions will be planted each year at Griffin to provide a continuous regeneration with those accessions from which seed are collected for more than one growing season.
- 2. Submit 51 PI=s, that have critically low levels of seed, for embryo rescue methods. Plantlets produced will be grown in greenhouse and/or field for seed regeneration.
- 3. Pearl Millet Collection (*Pennisetum glaucum*)

- a. One hundred PI-s of pearl millet with aged and/or low seed quantities will be grown for seed regeneration at the ARS unit at St. Croix, V.I., and the seed harvest will be received and processed for storage at Griffin.
- 4. Fiber Crop Collection (Kenaf, *Hibiscus cannabinus*; Roselle, *H. sabdiriffa*)
 - a. Thirty to 50 PI=s of kenaf and /or roselle will be regenerated in Mexico at the Tecoman Winter Nursery.
- 5. Bamboo Collection
 - a. The bamboo collection will be maintained in the established field plots at the ARS Fruit and Nut Research Unit, Byron, GA. Emphasis will be placed on adequate irrigation, weed control, and fire ant eradication.
- 6. Descriptor notes will be collected in a format acceptable for entry into GRIN.

PUBLICATIONS:	
See attachment.	
APPROVED:	
/s/ D. LaBonte	2/25/2000
D. LaBonte, Chair, Technical Committee	Date
/s/ G.F. Arkin	3/3/2000
G. F. Arkin, Administrative Advisor	Date

PUBLICATIONS:

<u>Alabama</u>

Jing Yu. 1999. Inheritance and variation of isozyme loci in red clover (*Trifolium pratense* L.). M. Sci. Thesis, Auburn University, Auburn, Alabama.

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Arkansas

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Florida

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Georgia

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Hawaii

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Kentucky

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Louisiana

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La Bonte, D.R., H.F. Harrison, and C.E. Motsenbocker. 1999. Sweetpotato clone tolerance to weed interference. HortScience 34:229-232.

Mississippi

Garcia, M., and C. E. Watson. 1999. Lodging resistance in sweet corn. So. Branch American Society of Agronomy Annual Meeting, Memphis, TN. 31 Jan. - 3 Feb. 1999. So. Branch Amer. Soc. Agron. Abstr. p.6.

Gourley, L. M., C. E. Watson, and A. S. Goggi. 1998. Bird-resistant grain sorghum A- and B-line inbreds released as germplasm. Inter. Sorghum Millets Newslett. 39:73.

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North Carolina

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